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Efficacy and safety of simultaneous rTMS–tDCS over bilateral angular gyrus on neuropsychiatric symptoms in patients with moderate Alzheimer's disease: A prospective, randomized, sham-controlled pilot study

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ABSTRACT

Background: Treating neuropsychiatric symptoms (NPS) in Alzheimer's disease (AD) remains highly challenging. Noninvasive brain stimulation using repetitive transcranial magnetic stimulation (rTMS) or transcranial direct current stimulation (tDCS) is of considerable interest in this context.
Objective: To investigate the efficacy and safety of a novel technique involving simultaneous application of rTMS and tDCS (rTMS–tDCS) over bilateral angular gyrus (AG, P5/P6 electrode site) for AD-related NPS.
Methods: Eighty-four AD patients were randomized to receive rTMS–tDCS, single-rTMS, single-tDCS, or sham stimulation for 4 weeks, with evaluation at week-4 (W4, immediately after treatment) and week-12 (W12, follow-up period) after initial examination. Primary outcome comprising Neuropsychiatric Inventory (NPI) score and secondary outcomes comprising mini-mental state examination (MMSE), AD assessment scale-cognitive subscale (ADAS-cog), and Pittsburgh sleep quality index (PSQI) scores were collected and analyzed by a two-factor (time and treatment), mixed-design ANOVA.
Results: rTMS–tDCS produced greater improvement in NPI scores than single-tDCS and sham at W4 and W12 (both $P < 0.017$) and trended better than single-rTMS (W4: $P = 0.058$, W12: $P = 0.034$). rTMS–tDCS improved MMSE scores compared with single-tDCS at W4 ($P = 0.011$) and sham at W4 and W12 (both $P < 0.017$). rTMS–tDCS also significantly improved PSQI compared with single-rTMS and sham (both $P < 0.017$). Interestingly, rTMS–tDCS-induced NPI/PSQI improvement was significantly associated with MMSE/ADAS-cog improvement. tDCS- and/or rTMS-related adverse events appeared slightly and briefly.
Conclusions: rTMS–tDCS application to bilateral AG can effectively improve AD-related NPS, cognitive function, and sleep quality with considerable safety.

1. Introduction

Neuropsychiatric symptoms (NPS) are prevalent in patients with Alzheimer's disease (AD), particularly in those with moderate disease [1]. NPS are significantly associated with faster cognitive decline [2], higher healthcare costs [3], greater mortality and caregiver burden [4], as well as worse daily activities and earlier hospitalization [5]. Despite the high prevalence and adverse effects

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Abbreviations

AD	Alzheimer's disease
AG	Angular gyrus
ChEI	cholinesterase inhibitors
DMN	Default mode network
FPCN	Frontoparietal control network
LTP	Long-term potentiation
MMSE	Mini-mental state examination
MMSE	noninvasive brain stimulation
ADAS-cog	AD assessment scale-cognitive subscale
NPI	Neuropsychiatric Inventory
NPS	Neuropsychiatric symptoms
PSQI	Pittsburgh sleep quality index
RMT	Resting motor threshold
rTMS	repetitive transcranial magnetic stimulation
tDCS	transcranial direct current stimulation

of NPS, the underlying pathogenesis remains unclear. Various contributing factors and indirect mediators have been described, including biological factors (e.g., brain changes, comorbidities) that interact with psychological (e.g., personality, stress responses) or social (e.g., environmental changes, support networks) factors [6]. Treating NPS is highly challenging because of the multimorbidity, polypharmacy, and complex etiology. Current treatments include traditional pharmacological/nonpharmacological approaches, with limited efficacy for NPS [7]. Moreover, the U.S. FDA had warned in 2005 and 2008 that using atypical and conventional antipsychotics increases the risk of mortality in patients with dementia [8,9]. Therefore, there is an urgent need to develop new techniques to alleviate psychiatric symptoms or potentiate the existing therapies for NPS.

Repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) are the two most widely used noninvasive brain stimulation (NIBS) techniques [10]. Their efficacy has been demonstrated in several therapeutic clinical trials on neurological and psychiatric diseases. However, inconsistent results have been reported, and treatment failures may occur within studies owing to different stimulus parameters [11]. There is evidence of nonlinearity with increases in intensity or duration, suggesting that longer or more intensive stimulation uncertainly increases the efficacy of tDCS and rTMS [12,13]. Hence, there is a need to combine different modes of NIBS to optimize neuromodulation-induced clinical benefits. Our previous study demonstrated that the simultaneous application of low-frequency rTMS with cathodal tDCS at the motor cortex boosts inhibitory aftereffects [14]. rTMS and tDCS are simultaneously applied for the following reasons: first, TMS is based on the principle of electromagnetic induction that can cause the depolarization of neuronal membranes and initiation of action potentials. tDCS uses a constant weak electric current (generally 1–2 mA) to modulate specific brain areas by bringing the membrane potential of neurons closer to (anodal tDCS) or farther from (cathodal tDCS) the action potential threshold. Combining rTMS with anodal tDCS may enhance the neuronal firing rate to boost the stimulation efficacy. Second, repetitive brain stimulation could generate a prolonged depolarizing response and facilitate activity-dependent communications, which would strengthen the stimulated neural circuitry [15]. rTMS has better focality while tDCS is used to stimulate large-scale brain volumes with the aim of altering the excitability of more than one cortical component of the network responsible for heterogeneous symptoms [16]. Third, it has been proposed that the amount of neuronal Ca^{2+} influx caused by the stimulation protocol is a crucial factor explaining the nonlinearity of tDCS effects. A brief but large increase in Ca^{2+} triggers long-term potentiation (LTP) [17]. The simultaneous application of high-frequency rTMS with anodal tDCS is an efficacious enhancement method of cortical excitability and may promote the reorganization of the activity of functional networks and provide important opportunities for driving long-lasting positive changes in brain diseases [18]. Therefore, in the present study, we introduced a novel protocol to preliminarily investigate whether simultaneous stimulation with tDCS and rTMS on the same target could enhance synergistic effects and improve AD-related NPS.

As a core node of the default mode network (DMN) and a potential convergence zone in AD, the angular gyrus (AG) has extensive connections with the medial prefrontal cortex, temporal cortex, precuneus, and superior and inferior frontal gyrus [19]. Patients with AD show a decline in the gray matter volume and functional coupling of bilateral AG [20]. Decreased functional connectivity has also been detected between the left AG and bilateral inferior parietal lobules, dorsolateral prefrontal, and lateral temporal cortices in patients converting from mild cognitive impairment to AD [21]. Our previous study showed that applying high-frequency rTMS over bilateral AG can improve cognitive function and frontal–parietal long-range functional connectivity in patients with AD [20]. These findings indicate that the AG is a potential target for improving cognitive function.

A recent study showed that atrophy in patients with clinical diagnosis of AD is functionally connected to the brain regions of the mesial temporal lobe, precuneus cortex, and AG [22]. Atrophy network mapping performed to define symptom-specific networks revealed that impaired memory was localized in the mesial temporal lobe, and delusions were localized in orbitofrontal cortex and AG. Moreover, lesion network mapping revealed lesions in the left retrosplenial cortex, which cause delusional misidentification [23]. Compared with healthy individuals, subjects with obsessive–compulsive disorder were found to have an abnormal resting-state spontaneous brain activity in the right AG and left middle frontal gyrus [24]. Furthermore, bifrontal electroconvulsive therapy altered the regional homogeneity and functional connectivity of the left AG in subjects with major depressive disorder [25]. Disruption in DMN has also been implicated in numerous neuropsychiatric disorders [26]. Therefore, we intended to investigate whether stimulating the AG to promote the intrinsic connectivity networks can improve the AD-related NPS.

Recent studies have demonstrated that brain function can be modulated by rhythmic TMS pulses because they regulate brain oscillations by resetting the ongoing oscillatory activity [27,28]. Neural circuits producing gamma rhythms are essential for amyloid clearance that occurs via increased microglial activity, which reduces synaptic toxicity [29]. A preclinical animal study has shown that an exogenously induced increase of gamma oscillations (specifically at 40 Hz) promotes microglial activation and causes subsequent reduction of amyloid-β (Aβ) and p-tau depositions in a mouse model of AD [30]. A pivotal preclinical study also found that 40-Hz sound stimulation can improve spatial recognition memory and reduce Aβ deposition in the auditory cortex and hippocampus in AD model rats [31]. In addition, our previous study showed that applying 40-Hz rTMS over bilateral AG modulating gamma-band oscillations effectively improved cognitive function in patients with probable AD [20]. On the basis of these findings, we applied high-frequency 40-Hz rTMS to modulate neuronal activity in the gamma-band.

In the present study, we present a novel protocol using simultaneous electro/magnetic stimulation on bilateral AG to explore the efficacy and safety of dual-modal stimulation (rTMS–tDCS) versus

single-tDCS/rTMS or sham stimulation for AD-related NPS. We also analyzed the subdomains of NPS to identify symptoms responsive to this new protocol.

2. Material and methods

2.1. Participants

The study protocol was approved by the Medical Research Ethics Committee of Xuanwu Hospital, Capital Medical University, China, and complied with the Declaration of Helsinki. Written informed consent was obtained from all patients or their surrogates before the experiment. A total of 84 right-handed patients (38 men and 46 women) diagnosed with probable AD recruited from the Department of Neurology of Xuanwu Hospital were included in this prospective, randomized, sham-controlled study (Fig. 1). The details of the sample size calculation was shown in Supplementary file 1.

All patients underwent a detailed history-taking, physical examination, and structural neuroimaging to exclude brain disorders other than AD at baseline. The inclusion criteria were an age of 60–90 years, probable AD diagnosed by the 2011 National Institute on Aging-Alzheimer's Association (NIA-AA) guidelines [32], having AD-related NPS, Clinical Dementia Rating score of 2, mini-mental state examination (MMSE) score of 10–20, Geriatric Depression Scale score of ≤8, Hachinski Ischemic Scale of <4, ≥5th-grade education, and able to cooperate with experiments. The exclusion criteria were patients with other neurological diseases or severe diseases of the heart, liver, lung, and kidney; alcohol and drug abuse; and metal implants, such as cardiac stents and pacemakers. Patients were randomized in a 1:1:1:1 ratio to receive rTMS–tDCS, single-tDCS, single-rTMS, or sham stimulation. Randomization code lists were computer-generated by unrelated collaborators using block randomization algorithm with a block size of 4, and sealed envelopes were prepared and saved. Eligible patients were first determined, and then the sealed envelopes were opened in the specified order to identify the patient group. Physicians-in-charge maintained the randomization codes, and stakeholders (including subjects, evaluators, and statisticians) were unaware of treatment allocations until statistical analysis reports were generated on June 7, 2022. Independent operators performed the brain stimulation procedure.

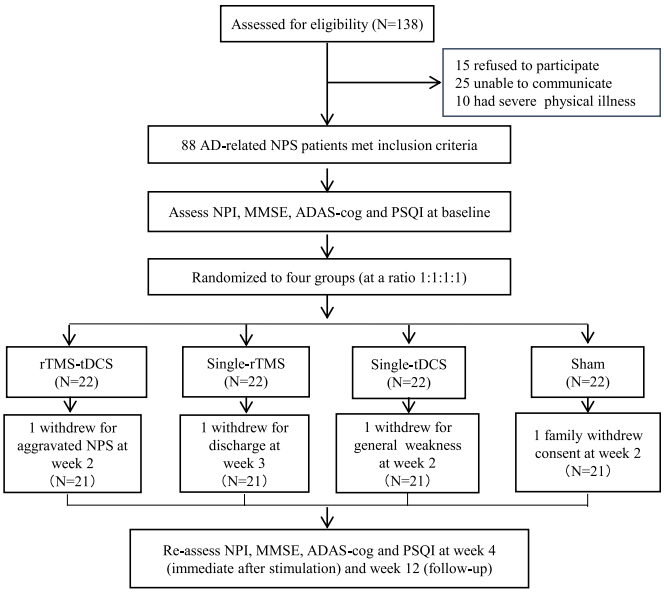

Fig. 1. Title: Flowchart of the progression of the participants through the study.
Caption: AD: Alzheimer's disease. NPS: Neuropsychiatric Symptoms. NPI: the Neuropsychiatric inventory. MMSE: Mini-Mental State Examination. ADAS-cog: AD Assessment Scale-cognitive subscale. PSQI: Pittsburgh Sleep Quality Index.

Patients taking memantine and cholinesterase inhibitors (ChEI) for at least 90 days before enrollment were asked to maintain a stable dosage throughout the study. All patients continued their usual medication and personalized daily care during the study. Limited doses of short-acting benzodiazepines or antipsychotics were permitted, but not more than three times per week.

2.2. Experimental protocol

Both electrical and magnetic stimulation was delivered by an Electromagnetic Stimulator (Tianjin Timus Medical Technology Co. LTD, Tianjin, China). During the experiment, each patient wore earplugs and reclined in a comfortable chair with the elbows slightly flexed. Operators were instructed to closely monitor the patients and prevent them from moving their heads during the intervention. All patients were treated on alternate days, three times a week for 4 consecutive weeks. A treatment schedule was prepared for each participant, and operators conducting the intervention were asked to sign an investigation form after each treatment to ensure study compliance.

2.2.1. rTMS application

Resting motor threshold (RMT) of the left abductor pollicis brevis was measured before the first treatment using a standard figure-of-eight coil connected to the Electromagnetic Stimulator. RMT was defined as the lowest stimulus intensity that triggered at least five motor-evoked potentials of >50 μV amplitude in 10 trials at rest [33]. The coil was tangent to the scalp, with the handle at a 45° angle to the midline. The location of AG stimulation was determined by the 10-10 International EEG System, corresponding to the P5/P6 region of the scalp. The rTMS coil overlapped with the anodal tDCS on the P5/P6 region of the EEG scalp as shown in Fig. 2. The coil was held stable over the AG using a mechanical arm and adjusted as necessary. A high-frequency 40-Hz rTMS at 90% RMT intensity was delivered to the AG (diameter, 70 mm; peak magnetic field, 2.0 T). The stimulation pattern is shown in Fig. 3. The coil we used for sham stimulation was a 70-mm double air diaphragm SHAM Coil supplied by the same company, which produced a sound that was as loud as that of real stimulation. When dissipated, the magnetic field stimulates the surface of the scalp but does not penetrate through the skull and down into the cortex. Sham stimulation followed the same paradigm as active stimulation, helping us to better examine the therapeutic effects of real magnetic stimulation.

2.2.2. tDCS application

A pair of saline-soaked, conventional electrodes delivered 2-mA direct current, the same size as those used in the study by Han et al. [14]. To avoid retinal phosphenes, the current was ramped up/down during the initial/final 25 s. The anode was applied to the AG (P5/P6, 10-10 International EEG System), and the cathode was applied to the contralateral prefrontal area (Fp2/Fp1, 10-10 International EEG System). After stimulation of the left AG for 15 min, the anode was moved to the right AG, and the cathode was switched from Fp2 to Fp1 for another 15 min. Sham tDCS comprised the ramping procedure (25-s fading in/out) at the beginning of the stimulation to provide the same initial sensation of active tDCS (Fig. 2).

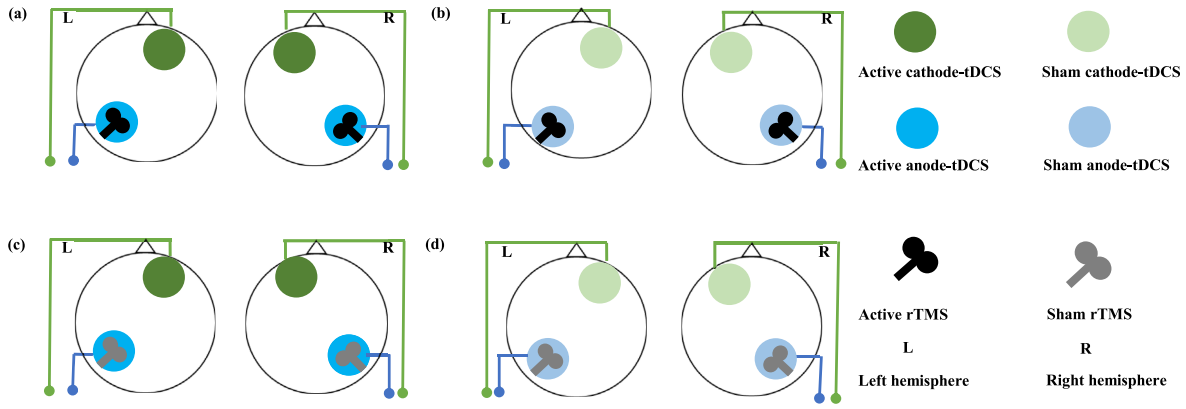

Fig. 2. Title: Experimental protocol.
Caption: All recruited patients received one of four stimulation groups: (a) rTMS-tDCS group, as the main experiment group, simultaneously received active rTMS and active anode-tDCS to bilateral angular gyrus (AG, P5 or P6 electrode site), while active cathode-tDCS over contralateral prefrontal area (Fp1 or Fp2 electrode site); (b) Single-rTMS group, simultaneously received active rTMS and sham tDCS; (c) Single-tDCS group, received active tDCS (anode to AG, cathode to contralateral prefrontal area) and sham rTMS; (d) Sham group, received sham rTMS and sham tDCS. The rTMS coil was overlapped with anodal tDCS on the P5/P6 region of the EEG scalp. rTMS: repetitive transcranial magnetic stimulation. tDCS: transcranial direct current stimulation. AG: angular gyrus.

2.3. Assessments

NPS were evaluated using the Neuropsychiatric Inventory (NPI, including 12 domains) [34]. The total score of each domain is calculated by multiplying the frequency score (1–4) by the severity score (1–3), and the sum of the scores of 12 domains is the NPI total score, ranging from 0 to 144 points, with higher scores indicating more pronounced symptoms.

Cognitive function was evaluated using MMSE and AD Assessment Scale-cognitive subscale (ADAS-cog). Sleep quality was evaluated using the Pittsburgh sleep quality index (PSQI). Safety was assessed by the occurrence and severity of adverse events, which were reported immediately and handled by experienced physicians.

All evaluations were performed by trained research assistants. The primary outcome measure was the NPI score, and the secondary outcomes comprised MMSE scores, ADAS-cog scores, PSQI, and adverse events. All evaluations were conducted at baseline, after 4 weeks of stimulation (W4), and 12 weeks after the initiation of intervention (follow-up, W12).

2.4. Statistical analysis

Statistical analysis was conducted using SPSS 25.0 (SPSS Inc., IL, USA) and GraphPad Prism 8. One-way ANOVA was performed for continuous variables, and the chi-square test was performed for categorical variables at baseline. The primary and secondary outcomes were analyzed by a two-factor (time and treatment), mixed-design ANOVA. The Shapiro–Wilk test was conducted to evaluate normality, which revealed normal distribution for all continuous variables at three measures. The sphericity of the set of variables was evaluated using the Mauchly test, and, when it was violated, the Greenhouse–Geisser correction was used. The effect size of the mixed-design ANOVA was determined using partial eta squared (ηp^2). Pairwise comparisons were performed three times for between-group (rTMS–tDCS vs. single-rTMS/single-tDCS/sham) and two times for within-group (W4/W12 vs. baseline) for NPI,

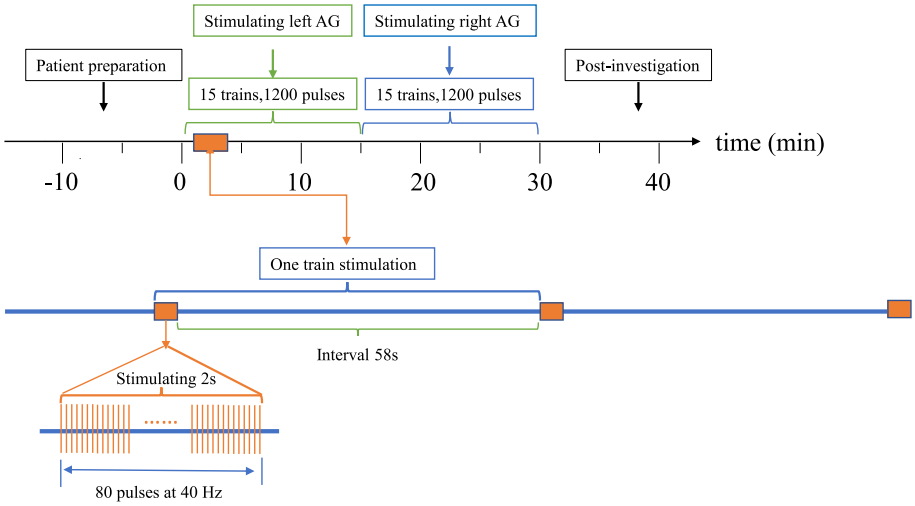

Fig. 3. Title: Stimulation pattern of rTMS.
Caption: Before each stimulation, each patient was prepared to wear earplugs and matched to rTMS coil in a suitable position. Daily rTMS stimulation time lasted 30-min (left 15-min followed by right 15-min). One train consisted of stimulating 2-s and interval 58-s at 40 Hz. Unilateral AG performed with 15 trains and 1200 pulses. After per-treatment, operators conducting the intervention were asked to investigate the participants if they had some adverse effects and record them in a report form. AG: angular gyrus.

MMSE, ADAS-cog, and PSQI scores. P < 0.017 (0.05/3) for between-group and P < 0.025 (0.05/2) for within-group comparisons were considered to be statistically significant after Bonferroni correction. Pearson's correlation analysis was used to examine the relationship between NPI/PSQI and MMSE/ADAS-cog scores. Except for multiple comparison correction data, the significant level was set at P < 0.05.

3. Results

Of 138 patients with probable AD screened for eligibility, 88 were enrolled in the study. One patient in each group withdrew for different reasons, and the remaining 84 patients (21 in each group) completed the 4-week intervention and 8-week follow-up for efficacy analysis. At baseline, no statistically significant differences were observed in age, gender, education, duration of AD, drug usage, RMT, and scores of NPI, MMSE, ADAS-cog, and PSQI (Table 1).

3.1. Primary outcomes

rTMS–tDCS resulted in greater improvement than single-tDCS and sham in NPS (decrease in NPI total score) at W4 and W12 (all P < 0.017) and elicited a trend toward larger improvement than single-rTMS (W4: P = 0.058, W12: P = 0.034). In the within-group comparisons, rTMS–tDCS, single-rTMS, and single-tDCS resulted in greater improvement in NPS at W4 and W12 than that at baseline (all P < 0.025), with no significant difference observed in sham treatment (Fig. 4).

Subscores of NPI were also evaluated. rTMS–tDCS led to a larger improvement in sleep disturbance than single-rTMS (W4: P = 0.017, W12: P = 0.013) and sham at W4 and W12 (W4/W12: both P < 0.001), with no statistical difference compared with single-tDCS (W4: P = 0.075, W12: P = 0.027). In the within-group comparison with baseline, sleep disturbance was significantly improved at W4 by rTMS–tDCS (P = 0.012) and single-tDCS (P = 0.003). Moreover, rTMS–tDCS resulted in greater improvement than sham in apathy (W4: P < 0.001, W12: P = 0.002), but no significant difference was observed between rTMS–tDCS and single-tDCS/rTMS (both P > 0.017). Apathy scores within the rTMS–tDCS group were significantly improved from baseline to W4 and W12 (W4: P < 0.001, W12: P = 0.001). No statistical difference was found in other subscores of NPI between and within groups.

3.2. Secondary outcomes

MMSE scores, and not ADAS-cog scores, were significantly improved by rTMS–tDCS compared to those by single-tDCS at W4 (P = 0.011) and sham at W4 and W12 (W4: P < 0.001, W12: P = 0.002), without statistical differences when compared with single-rTMS (W4: P = 0.045, W12: P = 0.056). In the within-group comparison with baseline, rTMS–tDCS produced significant improvement in MMSE scores at W4 and W12 (W4/W12: both P < 0.001) and ADAS-cog scores at W4 (P = 0.012), whereas single-rTMS/tDCS improved only MMSE scores at W4 (P < 0.025) (Table 2).

Furthermore, rTMS–tDCS led to a larger improvement in sleep quality (decreased PSQI scores) than single-rTMS and sham at W4 and W12 (both P < 0.017). No significant difference was observed between rTMS–tDCS and single-tDCS (W4: P = 0.039, W12: P = 0.021). In the within-group comparison with baseline, there was a significant improvement produced by rTMS–tDCS and single-tDCS at W4 and W12 (all P < 0.001), but no such finding was observed with single-rTMS and sham treatment (Fig. 5).

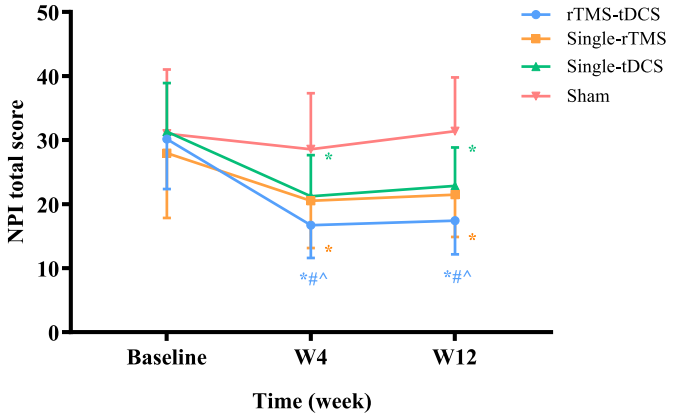

Fig. 4. Title: Comparisons of NPI total score among four groups at three measurements.
Caption: Error bars represent standard deviation. After Bonferroni correction, P < 0.017 (0.05/3) between-group and P < 0.025 (0.05/2) within-group were considered significant. *P < 0.025 vs. baseline. #P < 0.017 vs. Single-tDCS. ^P < 0.017 vs. Sham. Abbreviations: NPI: the neuropsychiatric inventory; rTMS: repetitive transcranial magnetic stimulation; tDCS: transcranial direct current stimulation; W4: after 4 weeks stimulation; W12: follow-up at week 12.

3.3. Correlation of NPI or PSQI score with MMSE and ADAS-cog scores

At baseline, there was no significant correlation between the total scores of NPI/PSQI and MMSE/ADAS-cog among the four groups (P > 0.05). After 4-week treatment, the decreased NPI or

Table 1
Demographic and clinical characteristics at baseline.

Variables	rTMS-tDCS (N = 21)	Single-rTMS (N = 21)	Single-tDCS (N = 21)	Sham (N = 21)	P value
Female, n (%)	9 (42.9%)	13 (61.9%)	13 (61.9%)	11 (52.4%)	0.549#
Age (years)	79.33 ± 6.24	76.86 ± 6.07	77.10 ± 6.88	75.33 ± 5.73	0.231*
Education (years)	12.43 ± 4.11	13.05 ± 3.67	10.86 ± 4.61	11.24 ± 4.39	0.303*
Duration (years)	3.52 ± 1.63	3.52 ± 1.67	3.93 ± 1.81	3.88 ± 2.01	0.811*
Basic assessments					
NPI	30.14 ± 7.77	27.95 ± 10.13	31.33 ± 7.59	31.00 ± 10.03	0.613*
MMSE	14.52 ± 3.04	13.52 ± 2.94	13.29 ± 3.45	15.05 ± 3.12	0.230*
ADAS-cog	41.62 ± 8.57	40.19 ± 7.81	42.43 ± 5.64	38.29 ± 9.59	0.365*
PSQI	11.38 ± 2.94	11.24 ± 2.93	11.48 ± 2.87	11.00 ± 2.45	0.951*
Drug usage, n (%)					
Memantine	16 (76.2%)	16 (76.2%)	19 (90.5%)	16 (76.2%)	0.574#
ChEIs	8 (38.1%)	9 (42.9%)	11 (52.4%)	6 (28.6%)	0.463#
RMT (%)	45.21 ± 6.87	44.67 ± 7.37	44.34 ± 7.77	46.76 ± 8.11	0.738*

Notes: Data are presented as (mean ± SD) or counts (%). NPI: the Neuropsychiatric Inventory; MMSE: Mini-mental State Examination; ADAS-cog: AD assessment scale-cognitive subscale; PSQI: Pittsburgh Sleep Quality Index; rTMS: repetitive transcranial magnetic stimulation; tDCS: transcranial direct current stimulation; ChEIs: Cholinesterase Inhibitors. RMT: Resting motor threshold. *: The p value was obtained by one-way ANOVA. #: The p value was obtained using Pearson χ^2 test.

Table 2
Results of cognitive function assessment among four groups at three assessments (W0, W4 and W12).

Scales	Groups	Times (mean ± SD)			P-value		
		W0	W4	W12	W4 vs. W0	W12 vs. W0	Group × Time
MMSE	rTMS-tDCS (21)	14.52 ± 3.04	19.38 ± 3.58#^	17.14 ± 3.42^	<0.001*	<0.001*	<0.001
	Single-rTMS (21)	13.52 ± 2.94	17.14 ± 3.44	15.14 ± 3.17	0.001*	0.094	
	Single-tDCS (21)	13.29 ± 3.45	16.57 ± 3.20	14.67 ± 3.26	0.003*	0.190	
	Sham (21)	15.05 ± 3.12	15.24 ± 2.93	13.90 ± 2.98	0.840	0.232	
ADAS-cog	rTMS-tDCS (21)	41.62 ± 8.57	34.14 ± 9.72	37.33 ± 9.12	0.012*	0.124	<0.001
	Single-rTMS (21)	40.19 ± 7.81	35.95 ± 8.88	38.71 ± 8.23	0.108	0.554	
	Single-tDCS (21)	42.43 ± 5.64	38.57 ± 6.55	40.67 ± 5.83	0.047	0.326	
	Sham (21)	38.29 ± 9.59	38.33 ± 10.19	39.95 ± 10.32	0.988	0.591	

Notes: #P < 0.017 vs. Single-tDCS; ^P < 0.017 vs. Sham. *P < 0.025 vs. baseline. After Bonferroni correction, P < 0.017 (0.05/3) between-group and P < 0.025 (0.05/2) within-group were considered significant. MMSE: Mini-mental State Examination; ADAS-cog: AD assessment scale-cognitive subscale; rTMS: repetitive transcranial magnetic stimulation; tDCS: transcranial direct current stimulation; W0: at baseline; W4: after stimulation at week 4; W12: follow-up at week 12.

PSQI score induced by rTMS–tDCS was significantly associated with improved MMSE scores ($r^2 = -0.532$, P = 0.013 for NPI with MMSE; $r^2 = -0.445$, P = 0.043 for PSQI with MMSE). At the 12-week follow-up, the improved NPI score produced by rTMS–tDCS was significantly associated with the improved ADAS-cog score ($r^2 = 0.549$, P = 0.010), whereas the deterioration of NPI score in the sham group was significantly associated with that of ADAS-cog score ($r^2 = 0.463$, P = 0.034).

3.4. Safety

During the intervention, participants experienced mild and transient headaches (7/84, 8.33%), scalp burns (6/84, 7.14%), and scalp numbness (6/84, 7.14%). However, there was no significant difference in the incidence of side effects among the four groups. Scalp burns were mild and improved significantly after the application of emollient oil, and headaches and scalp numbness were transient at the beginning of stimulation and recovered soon after the discontinuation of stimulation (Table 3).

4. Discussion

Simultaneous application of rTMS with tDCS (rTMS–tDCS) to regulate the function of AG as the default network core node resulted in a greater improvement in NPS than single-tDCS and sham treatment, with a trend toward a larger improvement than that by single-rTMS. Further, this novel technique induced greater improvement in cognitive function than single-tDCS and sham treatment as well as in sleep quality than single-rTMS and sham treatment. Interestingly, the improvements in NPS or sleep quality induced by rTMS–tDCS were significantly associated with cognitive improvement. This effect was noticed after 4 weeks of treatment and persisted until 8 weeks of follow-up with considerable safety, suggesting that rTMS–tDCS is safer and more effective than unimodal or sham stimulation for AD-related NPS.

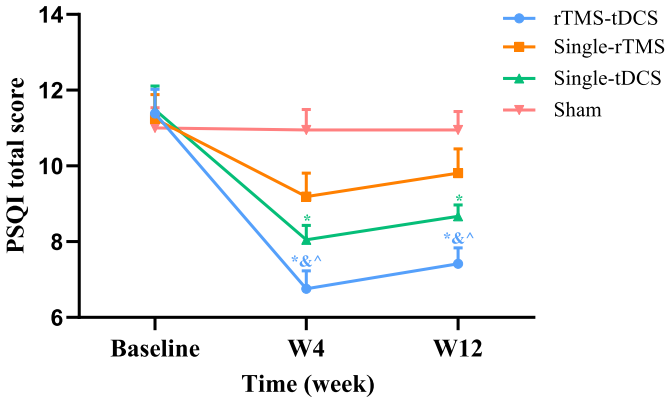

Fig. 5. Title: Comparison of PSQI total score among 4 groups at three measurements. **Caption:** Error bars represent standard error of mean. After Bonferroni correction, P < 0.017 (0.05/3) between-group and P < 0.025 (0.05/2) within-group were considered significant. *P < 0.025 vs. baseline. &P < 0.017 vs. single-rTMS. ^P < 0.017 vs. Sham. Abbreviations: PSQI: Pittsburgh Sleep Quality Index; rTMS: repetitive transcranial magnetic stimulation; tDCS: transcranial direct current stimulation; W4: after 4 weeks stimulation; W12: follow-up at week 12.

The neural mechanisms underlying this antipsychotic effect on AD-related NPS by rTMS–tDCS are not entirely clear. One possibility is that rTMS induces a brief but large increase in Ca^{2+} that induces LTP to enhance the synergistic effect of tDCS by modulating abnormal networks involved in AD-related NPS. Increasing evidence has indicated that NPS are caused by dysfunction in specific networks in AD, such as affective symptoms and apathy that are highly correlated with reduced functional connectivity of the frontoparietal control network (FPCN) [35]. DMN is involved in integrating self-referential information into conscious perception [36]. Recent imaging studies have shown that unconstrained DMN activity results in random formation of connections linking strong autobiographical correlates to trivial stimuli, thus resulting in hallucinations, delusions, and functional neurological disorders [37]. The decoupling of DMN from FPCN provides a functional substrate for psychosis onset [38]. The AG is a core hub of DMN and a vulnerable lesion in patients with AD. The atrophy and hypofunction of AG are involved in altered DMN activity and/or connectivity across intrinsic networks coactivation or deactivation [39], as well as alterations in information output to the prefrontal cortex, which may play a vital role in the development of psychiatric symptoms. Furthermore, a previous study revealed that improvement in cognitive function is a therapeutic strategy for AD-related NPS [5]. Consistent with this finding, our study demonstrated that the improved NPI score induced by rTMS–tDCS was significantly associated with the improved MMSE score. Therefore, the application of rTMS–tDCS to the AG may exert a therapeutic effect on NPS by promoting the functional integration of DMN and FPCN and improving cognitive function.

Our results demonstrated that rTMS–tDCS greatly improved cognitive function compared with single-tDCS and sham treatment, without any statistical difference compared with single-rTMS. This may because of the small sample size; another reason may be that regulating the brain oscillatory activity plays an important role in improving cognitive function. Gamma-band oscillations (30–80 Hz) are most closely associated with high-order cognitive function [40]. Studies on synaptic plasticity based on spike timing have indicated that gamma oscillations can accurately and rapidly establish synchronous neuronal activity between pre- and

Table 3
The incidence of side effects in the four groups.

Side effects (n, %)	rTMS-tDCS (N = 21)	Single-rTMS (N = 21)	Single-tDCS (N = 21)	Sham (N = 21)	χ^2	P-value*
Headache	3 (14.3%)	2 (9.5%)	2 (9.5%)	0	3.148	0.502
Scalp burns	3 (14.3%)	0	3 (14.3%)	0	5.753	0.076
Scalp numbness	3 (14.3%)	0	3 (14.3%)	0	5.753	0.076

Notes : *p-value was obtained using Fisher's exact test. rTMS: repetitive transcranial magnetic stimulation; tDCS: transcranial direct current stimulation.

postsynaptic neurons within intervals of 10–30 ms [41], strengthen the connections between neurons, and promote information transmission through the Hebbian rule [42,43]. Recently, 40-Hz gamma-rhythmic sensory stimuli (flicker and acoustic stimulation) have been used to improve cognitive function, providing an important advancement in the nonpharmacological treatment of cognitive impairment [29,31]. Furthermore, our recent research revealed that 40-Hz rTMS that produces gamma rhythms can significantly improve cognitive function in patients with mild AD [20]. Therefore, the application of 40-Hz rTMS may facilitate the synergistic effect of rTMS–tDCS on cognitive improvement.

We also found that rTMS–tDCS improved sleep quality better than single-rTMS, suggesting a synergistic effect of tDCS on rTMS in treating sleep disturbance. The possible mechanism underlying this beneficial effect is the application of long-range tDCS, with the anode over the AG (P5/P6) and the cathode over the prefrontal cortex (Fp1/Fp2). Previous research has demonstrated that anodal tDCS increases delta power in the temporoparietal region and cathodal tDCS increases delta and theta power in the frontal region, which may facilitate slow-wave sleep oscillatory activity at a long-range cortical network [44]. In addition, the long-range tDCS may be involved in parts of the top-down corticothalamic pathway of sleep–wake regulation [45]. Therefore, the application of tDCS to regulate more cortical and subcortical brain network activity to enhance slow-wave power may result in a better therapeutic effect of rTMS–tDCS on improving sleep disorder in patients with moderate AD.

Consistent with previous studies, our study also found that improved NPS and sleep quality induced by tDCS-rTMS were positively associated with improved cognitive function [46,47]. Despite the high rate of comorbidity between NPS/sleep disorder and cognitive impairment, the underlying neurobiological relationship remains unclear. The modern theory tends to consider NPS/sleep disorder and cognitive impairment as two distinct but related neuropsychiatric syndromes [48]. Consistent with this, our study revealed no correlation between NPS/sleep quality and cognitive function at baseline, but a significant correlation was detected after 4-week rTMS–tDCS stimulation. It is speculated that a pathophysiological relationship exists between NPS/sleep disorder and cognitive impairment, and shared neural circuits or brain networks may be involved in the neuromodulation of NPS/sleep disorder and cognition. However, further large-scale studies are required to investigate the relationship between NPS/sleep disorder and cognition.

This study has several limitations. First, to avoid high dropout rates, we recruited only patients with moderate AD who can cooperate with rTMS or tDCS, which may result in small sample size per group and introduce type 2 statistical errors, further resulting in some data without statistically significant difference. Second, the 12-session course for AD-related NPS therapy is relatively short. It would be extremely important to lengthen the duration of the therapeutic courses in the future. Third, we obtained only MMSE scores. MoCA may be more sensitive for detecting small improvements/declines in cognitive function. Data on MoCA would be important for consideration in future studies. Moreover, this study did not set other stimulation frequencies, such as 20, 10, and 1 Hz or theta-burst, to compare differences in therapeutic effects with 40 Hz. Therefore, further studies with larger sample sizes and longer observation periods on different stimulation frequencies are necessary.

5. Conclusions

Simultaneous rTMS–tDCS application as a novel stimulation technique is more effective than single-tDCS/rTMS in improving AD-related NPS, cognitive function, and sleep quality. Shaping the parameters in further studies may tentatively optimize the synergistic effects in an even better manner.

CRediT authorship contribution statement

Yueqing Hu: Conceptualization, Investigation, Methodology, Project administration, Visualization, Writing – original draft. **Yu Jia:** Investigation, Software, Validation, Data curation. **Ying Sun:** Methodology, Resources, Investigation. **Yan Ding:** Methodology, Resources, Investigation. **Zhaoyang Huang:** Methodology, Resources, Investigation. **Chunyan Liu:** Conceptualization, Investigation, Methodology, Project administration, Writing – review & editing. **Yuping Wang:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2022.11.009.

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